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Research Article

Comparative Studies of EDTA, Sodium Citrate and Aqueous Extract of *Triclisia Dictyophylla*

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Abstract: Some anticoagulants used in the laboratory for in vitro studies have some de-merits while others confer some changes on cellular components. Hence, the need to identify and characterize new anticoagulants. Aqueous extract of *Triclisia dictyophylla* was prepared and compared with EDTA and sodium citrate. 6mls of blood was collected from 200 human volunteers and dispensed 2mls each into EDTA, sodium citrate and 200mg of the extract. Standard methods were used to analyze the samples and the results were compared. The extract showed significant increase ($p<0.05$) in PCV, Hb, platelet count, ESR and plasma viscosity while WBC showed significant reduction when compared. The refined extract could possess better anticoagulation activity which maybe of importance in the laboratory for in vitro use.
Keywords: EDTA, sodium citrate, Triclisia dictyophylla, refined extract

INTRODUCTION

Anticoagulant is any substance which can be used to prevent blood coagulation [1]. Anticoagulants can prevent blood coagulation by catalyzing the action of antithrombin III (Antithrombin), chelating calcium ions or by forming complexes with calcium ion. Inhibition of thromboxane A_2 formation prevents platelet aggregation thus can also aid in anticoagulation. Dislodgement or mechanical degradation of fibrin clot can also bring about anticoagulation. EDTA and sodium citrate remove calcium, which is essential for coagulation. Calcium is either precipitated as insoluble oxalate (crystals of which may be seen in oxalated blood) or bound in a nonionized form. Heparin binds to antithrombin, thus inhibiting the interaction of several clotting factors.

EDTA is used for blood counts; sodium citrate is used for coagulation testing and erythrocyte sedimentation rate (ESR). For better long-term preservation of red cells for certain tests and for transfusion purposes, citrate is used in combination with dextrose in the form of acid-citrate-dextrose (ACD), citrate-phosphate-dextrose (CPD), or Alsever's solution. Anticoagulant mixtures are also used to compensate for disadvantages in each and to meet the needs of the analytic process [2, 3]. Any anticoagulant can be used for collecting blood for flow cytometry [4].

Anticoagulants can prevent blood coagulation by catalyzing the action of antithrombin III (Antithrombin), chelating calcium ions or by forming complexes with calcium ion. Inhibition of thromboxane A_2 formation prevents platelet aggregation thus can also aid in anticoagulation. Dislodgement or mechanical degradation of fibrin clot can also bring about anticoagulation. Anticoagulants can be used in vivo (as drugs) and in vitro (in blood collecting and processing).

Anticoagulant drugs (in vivo) are used to treat and prevent hyper coagulatory state and thrombotic disorders [1], and to prevent and treat stroke and transient ischaemic attack [5]. They are also given to prevent abnormal blood clotting after major surgery or during haemodialysis. The most commonly used ones are heparins, low-molecular weight heparins and the newer heparin derived-drugs such as tinzaparin, all of which are administered intraparentally. Warfarin, which is taken orally is derived from coumarin and exerts its anticoagulation effect by inhibiting vitamin K reductase which invariably prevents the gamma carboxylation of glutamic acid residue of factors II, VII, IX and X. Heparins exert their anticoagulation activity by catalyzing the penta saccharide residue of anti thrombin III (AT-III) and facilitating its antithrombin effect. Inhibition of protein C and protein S by a natural action of Antithrombin helps to prevent hypercoagulatory state. Deficiency of these proteins has been demonstrated in factor V Leiden and has been shown to be responsible for hypercoagulatory state in this category of patients [1].

Triclisia dictyophylla is a member of the family Menispermaceae (Moon seed).The Menispermaceae is a temperate to tropical family of around 70 genera (including Triclisia) and 450 species of dicotyledenous tropical flowering vines with twining stems and a few herbs, shrubs and trees. Leaves are alternate and simple, but may be palmately veined and often lobed. *Triclisia dictyophylla* is a medicinal plant that is indigenous to Africa. It has been shown to possess in vivo

anticoagulation activity [6, 7] and anti-microbial activity [8] possibly justifying its use in the treatment of several ailments like oedema, anaemia and spasm [9].

In laboratory practice, some anticoagulants are reported to cause deleterious effects on blood cellular elements, while others confer unwanted bizarre colouration on cells [10]. An exploratory research effort towards identifying and characterizing new anticoagulants is worthwhile.

MATERIALS AND METHODS

Aqueous Extraction

Triclisia dictyophylla plants were uprooted from their natural habitat and allowed to air-dry for 4 days. The roots were chopped into bits and pounded in a wooden mortar and immersed in 2 litres of distilled water and left undisturbed for 48 hours. Filtration was done using Whatman's No 1 filter paper to obtain the brownish filtrate. The filterate was poured into the conical flask of rotary evaporator. The filterate was evaporated into a dry semisolid black-brown residue which was poured into an evaporating dish and put in an incubator at 60^0C for 3 days [11]. Solid extract was obtained, weighed and refrigerated.

Sample Collection

Two hundred (200) human volunteers were used for this study. Verbal consent was obtained prior to sample collection. 6 mls of blood sample was collected, 2 mls was transferred into plain container containing 200mg of extract of *Triclisia dictyophylla*. 2 mls each was transferred into EDTA and Sodium citrate bottles for analysis.

Sample Analysis

Plasma and Whole blood Viscosity [12]

Principle

The test is based on the comparison of the rate of flow of plasma/whole blood and distilled water under equal pressure and constant temperature.

Procedures

The 1 mL syringe was clamped on the retort stand in a vertical position. The well-mixed whole blood was suctioned by pulling the plunger of the syringe so that the plunger rises above the upper measuring line. The suction was then released by gently pulling out the plunger and the whole blood was allowed to flow through the barrel. A stop watch was started when the meniscus got to the upper measuring line, and the time required for the meniscus to pass the lower measuring line was determined, recorded and repeated twice. The whole blood was removed from the syringes and then rinsed twice with normal saline and dried. The syringe was reclamped and distilled water was determined the same way as whole blood.

Calculation of Results

$$\textit{Plasma Viscosity} = \frac{\textit{Flow time of plasma (secs)}}{\text{Flow time of D/water (secs)}}$$

$$\text{Whole blood viscosity} = \frac{\text{Flow time of whole blood (secs)}}{\text{Flow time of D/water (secs)}}$$

Erythrocyte Sedimentation rate (ESR) was analyzed using Westergren Method and Packed Cell volume (PCV) using microhaematocrit method. Haemoglobin concentration (Hb) was analyzed using cyanmethaemoglobin method. WBC and platelet were diluted with Turk's fluid and 1% ammonium oxalate respectively and counted using improved Neubauer counting chamber [13,14]. All samples were analyzed within six (6) hours of collection.

RESULTS AND DISCUSSION

Table 1:Extract of *Triclisia dictyophylla* compared with EDTA

Parameters	Test (extract)	Control (EDTA)	Pvalue
PCV (%)	37.05 ± 0.21	35.60 ± 0.21	p<0.05
Hb (g/dl)	13.14 ± 0.08	12.85 ± 0.07	p<0.05
WBC ($x10^9$/l)	6.98 ± 0.05	7.21 ± 0.05	p<0.05
PLT ($x10^9$/l)	261150.00 ± 4079.00	247750.00 ± 3634.00	p<0.05
NEUT (%)	50.30 ± 0.53	48.15 ± 0.56	p>0.05
LYM (%)	49.40 ± 0.50	48.45 ± 0.51	p>0.05
EOS (%)	1.80 ± 0.18	1.90 ± 0.16	p>0.05
MONO (%)	0.30 ± 0.03	0.30 ± 0.03	p>0.05

PCV – Packed Cell Volume; Hb – Haemoglobin; WBC – White Blood Cell count; PLT – Platelet count; Neut – Neutrophil; Lym – Lymphocyte; Eos – Eosinophil; Mono - Monocyte

Table 2:Extract of *Triclisia dictyophylla* compared with sodium citrate

Parameters	Test (extract)	Control (Sodium citrate)	Pvalue
ESR (mm/hr)	15.10 ± 0.37	13.45 ± 0.35	p<0.05
RPV (mPa.s)	1.27 ± 0.003	1.24 ± 0.003	p<0.05
RWBV (mPa.s)	1.62 ± 0.009	1.61 ± 0.009	p>0.05
PFC (g/l)	2.69 ± 0.05	2.53 ± 0.05	p<0.05

PCV – Packed Cell Volume; Hb – Haemoglobin; WBC – White Blood Cell count; PLT – Platelet count; Neut – Neutrophil; Lym – Lymphocyte; Eos – Eosinophil; Mono - Monocyte

The *in vitro* studies was done using *T. dictyophylla* as an anticoagulant at a concentration of 200mg/2ml of blood [6] and compared with EDTA and sodium citrate where appropriate. The packed cell volume (PCV) of the extract (37.05±0.21%) was significantly higher than the control (35.60±0.21%). This may be attributed to the fine particles of the extract. The erythrocyte sedimentation rate (ESR) of the extract (15.10±0.37mm/hr) was also higher compared to the control (13.45±0.35mm/hr).

Haemorheology is the science of deformation and flow of blood and its formed elements [15]. Haemorheological parameters determine the flow pattern of blood. Certain parameters are used to assess flow pattern and they include relative plasma viscosity, whole blood viscosity and plasma fibrinogen concentration. The plasma viscosity of the extract was 1.27±0.003m.pas and 1.24±0.003m.pas for the control. The relative whole blood viscosity of the extract and control were 1.62±0.009m.pas and 1.61±0.009m.pas respectively. The plasma fibrinogen concentration of the extract (2.69±0.05g/dl) was significantly higher than the control (2.53±0.05g/dl). These differences could be attributed to the active constituents of the extract. Much literature is not available in this regard so as to compare with previous works. The haemoglobin concentration showed a significant increase in the extract (13.14±0.08g/dl) compared to control (12.85±0.07g/dl). Such increase was recorded for haemoglobin in the *in vivo* studies[7] using albino Wistar rats. White blood cell count (WBC) was significantly lower in the extract than the control. The platelet count showed a significantly higher count in the extract (261150±4079 $x10^9$/l) than in the control (247750±3634 $x10^9$/l). Neutrophil, lymphocyte, eosinophil and monocyte all were not statistically significant when compared (p>0.05). These comparisms using extract of *Triclisia dictyophylla*, EDTA and sodium citrate showed little or no difference among them. This shows a promising prospect for its use for *in vitro* studies.

CONCLUSION

At a concentration of 200mg to 2ml of blood, the crude extract of *Triclisia dictyophylla* showed little or no difference when compared to EDTA and sodium citrate, which is very promising for laboratory use. The refined extract could possess better anticoagulation properties. We therefore recommend for further studies to properly identify the structure and other characteristics of this "anticoagulant" which we now refer to as "dictin".

REFERENCES

1. Famodu AA; Concise haemostasis and thrombosis. 2nd Edition, Ode-magba Scientist Publisher, Lagos, 2003: 153.
2. Narayanan S; The pre-analytic phase: an important component of Laboratory Medicine. American Journal of Clinical Pathology, 2000; 113: 429 – 457.
3. Tatsumi N, Miwa S and Lewis SM; Specimen collection, storage and transportation to the laboratory for haematological tests. International Journal of Haematology, 2002; 75: 261 – 268.
4. National Committee for Clinical Laboratory Standards; Clinical application of flow cytometry: quality assurance and immunophenotyping of peripheral blood lymphocytes. Document H43-A, NCCLS, Wayne, 199.
5. British Medical Association; Illustrated Medical Dictionary. Peters M (Ed). D.K. London, 2002: 40.
6. Azikiwe CC, Amazu LU, Unekwe PC and Nwosu PJC; Anticoagulation and antithrombotic effects of Triclisia dictyophylla (moon seed). Nigerian Journal of Natural Plants Medicine, 2007, 11: 29 – 31.
7. Ajugwo AO and Ezimah AC; In vivo studies of anticoagulation activity of Triclisia dictyophylla using albino wistar rats. Int. Journal of Pharmaceutical Sci. Inventions, 2013, 2(5): 37 – 40.
8. Ajugwo AO, Ezimah ACU, Awah FM, Mounmbegna PP, Azikiwe CC and Abouo AM; Phytochemical and antimicrobial properties of the aqueous root extract of *Triclisia dictyophylla*. Asian Journal of Medical Science, 2013, 4 (1): 15 – 20.
9. Spiff AL, Zabel V, Watsin WH, Zemaitis WA and Ateya AM; Constituents of West

African Medical Plant xxxx, Tridictyophylline, a new morphinan alkaloid from Triclisia dictyophlla. Int. Journal of Natural Products, 1981, 44 (2): 160 – 165.

10. Bain B J; Blood cells: A practical guide. Gower Publishers London, 1989.
11. Ajugwo AO; Studies on the isolation, extent and mechanism of anticoagulant and anti-microbial activity of the extract of *Triclisia dictyophylla*. Igbinedion University, Okada, 2013; 94 -95.
12. Reid HL and Ugwu AC; A simple technique for rapid determination of plasma viscosity. Nig. Journal of Physiological Science, 1987, 3: 45 – 48.
13. Cheesbrough M; Haematological tests. In: District laboratory practice in tropical countries. Part 2 Cambridge University Press U.K., 2000: 297.
14. Ingram GC; A suggested schedule for rapid investigation of acute haemostatic failure. Journal of Clinical Pathology, 1961, 14: 156 – 160.
15. Popel AS and Johnson PC; Microcirculation and hemorheology. Annual Review of Fluid Mechanics, 2005, 37: 43-69.